

Vestimentiferan on a Whale Fall

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Discovery of chemosynthetic communities associated with whale bones led to the hypothesis that whale falls may serve as stepping-stones for faunal dispersal between disjunct hydrothermal vents and cold seeps on the ocean floor (1). The initial observation was followed by a faunal inventory that revealed a diverse assemblage of microbes and invertebrates, supported by chemoautotrophic production, living in close proximity to whale remains (2, 3). To date, the conspicuous absence from whale falls of vestimentiferan tubeworms (a predominant constituent of eastern Pacific vent and seep habitats) has been a major objection to the stepping-stone hypothesis (4–5). We report the first evidence of a vestimentiferan tubeworm associated with a whale fall (Fig. 1). The tubeworm, *Escarpia spicata*, was identified by morphological criteria and DNA sequence data from a portion of the mitochondrial cytochrome oxidase C subunit I (COI) gene. Additionally, the bacterial endosymbiont in the tubeworm possessed a 16S rRNA gene that was similar to that of endosymbionts from vestimentiferans in sedimented cold-seep environments.

A chemosynthetic community associated with whale bones in the Santa Catalina Basin (SCB) off southern California (depth 1240 m, 33°12'N, 118°30'W) is the site of ongoing ecological studies (1, 2). We revisited the SCB site in May 1995 with the U.S. Navy's advanced tethered vehicle (ATV dive #118) and found a persistent community composed of filamentous sulfur bacteria (*Beggiatoa*

sp.), vesicomylid clams (*VesicomyalCalyptogena* spp.), mytilids (*Idas* sp.), limpets (*Pyropelta* spp., *Cocculina* sp.), snails (*Mitrella* sp.), and polynoid polychaetes (*Bathyrurila* sp.). We also recovered a single vestimentiferan tubeworm growing near the squamosal bone of the whale skeleton (Fig. 1). The tubeworm was collected and preserved in 95% ethanol once aboard the support ship. The worm was initially identified as *Escarpia spicata* on the basis of diagnostic morphological features (6), most notably the central, slightly curved spikelike structure on the obturacular face.

DNA sequence information from a mitochondrial gene verified the identity of the SCB specimen. A 639-bp portion of the COI gene from the SCB vestimentiferan was identical to that of an *Escarpia spicata* individual sampled from cold seeps (Transform Fault) in the Gulf of California (Table I). The COI sequence of the SCB specimen differed by 0.32% from those of *Escarpia spicata* from hydrothermal vents (Guaymas Basin) in the Gulf of California (Table I). COI sequence differences less than 0.4% are consistent with intraspecific levels of divergence in vestimentiferans (7). Intergeneric COI sequence divergence in the Vestimentifera ranges from 8.5%–21.3% (7).

The bacterial endosymbiont from the SCB *E. spicata* was phylogenetically compared to other endosymbionts as part of a separate study (8). Sequence information from a 1361-bp region of the 16S rRNA gene (Genbank accession number U77842) revealed that the endosymbiont is a member of the gamma subdivision of the Proteobacteria. The SCB endosymbiont was closely related (1.45% sequence divergence) to endosymbionts found in vestimentiferans that live in sedimented seep localities; its relationship to endosymbionts from vestimentiferans found in

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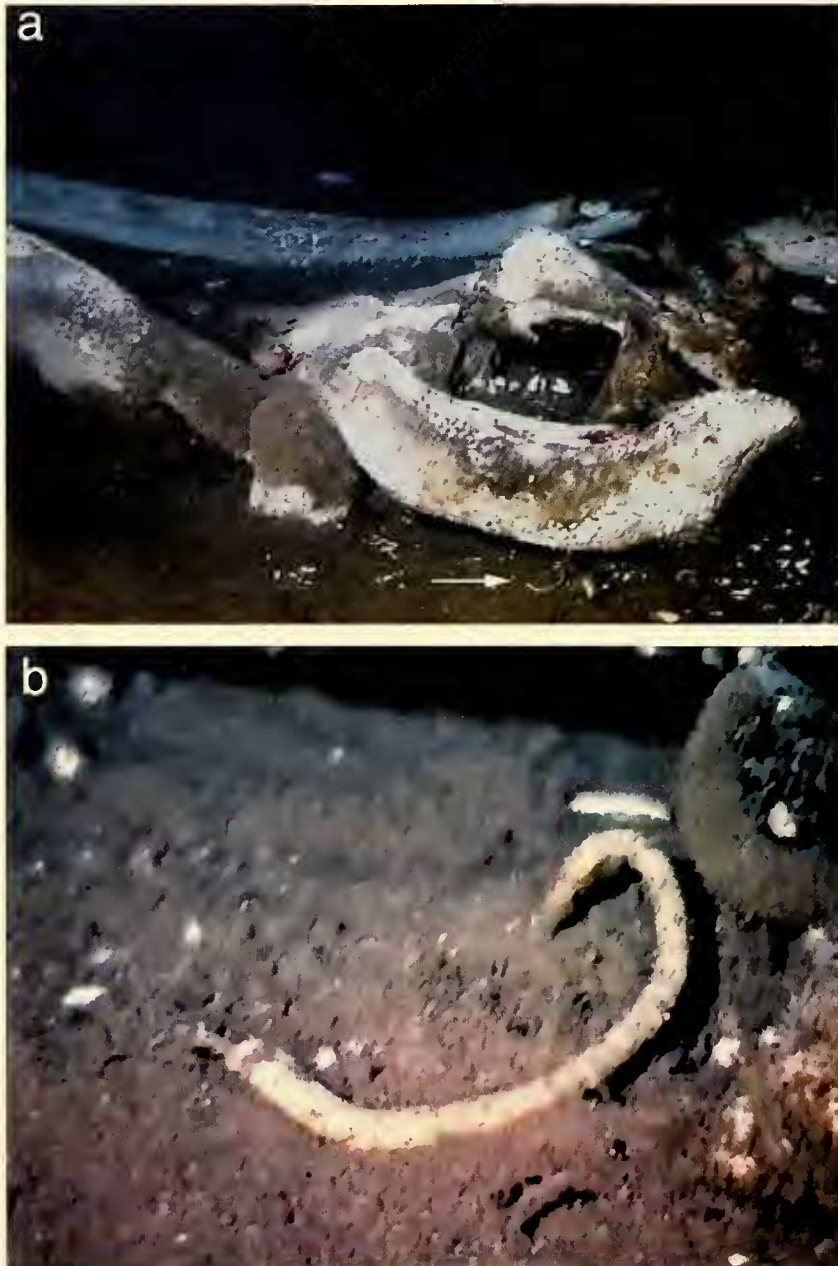


Figure 1. Skull bones of an 18-m baleanopterid skeleton, and associated chemoautotrophic community. (a) A broad view of the skull region with the first recorded adult vestimentiferan (arrow) at a whale fall (for scale, the squamosal bone in the right foreground is 80-cm long). The vestimentiferan plume (left) and posterior end of the tube (right) are visible in the magnified image (b). This individual was morphologically and genetically identified as *Escarpia spicata*. With the exception of *Lamellibrachia columna*, supplied by C. Cary (Univ. of Delaware), the specimens examined in this study were collected during oceanic cruises by one or more of the authors. DNA was extracted using the hexadecyl-trimethyl-ammonium bromide (CTAB) technique, modified from Doyle and Dickson (12). For the amplification of the mitochondrial COI gene; LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-ACTAAAAACCA-GTGGGACTTCAAAT-3' primers were used in standard conditions (13). To amplify symbiont and sequence the 16S rDNA, we used the universal bacterial primers 27F and 1492R (14); 27F: 5'-AGAGTTTGATCM-TGGCTCAG-3', and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' and a suite of internal primers (8). PCR products were purified and quantified on a video imaging system (Fotodyne Inc.), 100 ng of pure DNA was used as template for ABI Prism DNA sequencing reactions. The sequenced fragments were electrophoretically separated and detected using a Perkin Elmer ABI 373A DNA sequencer.

Table 1

Percent genetic distances between the *Escarpia spicata* individual from the Santa Catalina Basin whale fall and related vestimentiferans for a 639-bp portion of the mitochondrial COI gene

Species	Location	Collection Site ¹				Ref	Genetic Distance Matrix ²				
		Lat Long	Substrate, Community Type	Depth (m)	1		2	3	4	5	
<i>E. spicata</i>	Guaymas Basin	27°35'N 111°28'W	sedimented, cold seep	1653	7	—					
<i>E. spicata</i>	Guaymas Basin	27°00'N 111°25'W	basaltic, vent	2020	7	0.32	—				
<i>E. spicata</i>	Santa Catalina Basin	33°12'N 118°30'W	sedimented, whale fall	1240	this study	0.00	0.32	—			
<i>E. laminata</i>	W. Florida Escarpment	26°02'N 84°55'W	sedimented, seep	3243	8	0.32	0.32	0.32	—		
<i>Lamellibrachia columna</i>	Lau Basin Hine Hina	22°32'S 176°43'W	sedimented, seep	1859	15	14.87	14.87	14.87	15.53	—	

¹ Summary of collection information for all specimens analyzed.

² Distances are based on the Kimura 2-parameter model (16). Genbank accession numbers for these sequences are U74065, U74064, U84262, U74063, and U74061.

deep-sea hydrothermal vent habitats was more distant (4.5%).

Escarpia spicata occurs at cold-water sulfide seeps along the Louisiana Slope (9), southern California (type locality is Navy Fan, South San Clemente Basin) (6), Gulf of California (7), and in soft sediments near hydrothermal vents in the Guaymas Basin (7). Its presence in soft sediments near the Guaymas hot vents suggests that it may be an opportunistic species with a wide tolerance for habitat variation. Similarly, *Lamellibrachia barhami* is common in cold seeps in the northeastern Pacific but also occurs at new hot vents in Middle Valley (7). Vestimentiferan adults are sessile, but their larvae are free-swimming and may persist in the water column for as long as 3 weeks (10). Additionally, allozyme studies reveal that vestimentiferans have effective dispersal capabilities that maintain genetic continuity across vast distances (11). The potential for long larval life spans and the opportunistic settling habits of members of the Vestimentifera suggest that the SCB whale site is probably within the dispersal range of *E. spicata* larvae from the San Clemente Basin site.

The discovery of an adult vestimentiferan on the SCB whale fall suggests that such falls can support the recruitment and growth of at least one species of vestimentiferan. This finding, as well as the identification of seep and vent vesicomyids on whale falls, is consistent with the hypothesis that whale carcasses may serve as stepping-stones for seep and vent organisms (1). However, other models of metapopulation dynamics should also be considered, especially because we have not demonstrated the presence of a reproductive population of *E. spicata* on the SCB whale carcass. Careful analyses of the repro-

ductive potential and persistence of whale-fall fauna are necessary to ascertain whether these animals contribute to the propagation of chemosynthetic communities in the deep sea. Alternatively, whale-fall fauna may represent sink populations that are colonized from more stable chemosynthetic source communities at nearby seeps or vents.

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